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MALTA FEVER, WITH SPECIAL REFERENCE TO ITS DIAGNOSIS AND CONTROL IN GOATS.

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INTRODUCTION.

On the island of Malta there has been endemic for an indefinite period a febrile disease of the inhabitants, termed "Malta fever," also known as "Rock," "Mediterranean," or "undulant" fever, and vernacularly called "slow," "dust," or "goat" fever. This infection was so exceedingly prevalent among the British soldiers and sailors stationed on the island that in 1904 a commission was appointed by the British Government, under the supervision of an advisory committee of the British Royal Society, to investigate the possible sources of infection and advise methods for its control. The commission has since investigated the disease in all of its phases in a most exhaustive manner, and as early as 1905[1] was led to consider that the milk from the native goats was an important if not the main factor in the dissemination of Malta fever among human beings. It was found that the goats were not only susceptible to artificial infection, but that about 50 per cent of them acquired the disease naturally, and that the organisms producing the disease were eliminated in their milk and urine. It was then decided to investigate the milk of such infected though apparently healthy goats, with the result that in Malta 10 per cent of the goats were found to be eliminating the specific coccus in the milk, and this milk when fed to monkeys even for a day was able to produce typical attacks of Malta fever which ran a course parallel to that of the disease in man. The only logical conclusion which could be formulated from this work was that the Maltese goats were carriers of the virus of Malta fever and were one of the principal means of transmitting the disease to human beings through the ingestion of their milk. All the available evidence points to contaminated food as the vehicle by which these goats become infected with the virus of Malta fever. Furthermore, it has been shown that the urine of infected goats

[1] Report of the Commission on Mediterranean Fever, pt. 3, p. 2. London, 1905.

and of ambulatory cases in man at times contains the causative agent, *Micrococcus melitensis*, so that goats feeding on material that has come in contact with such urine (which is not at all infrequent by the usual method of handling these animals) are readily infected. Thus the frequency and the method of infection in goats are quite readily explained.

Other proofs of the transmission of Malta fever to man through the milk of infected goats are forthcoming from a number of experiences which have been noted since regulations were enforced in infected districts looking to the elimination of goat's milk as a source of infection. Thus the disease is said to have practically disappeared from Gibraltar following the removal of the infected goats. The affection does not occur among the convicts of the civil prison at Malta who are not allowed milk, but Malta fever is nevertheless present in the district where the prison is located. All fresh milk now being supplied to the soldiers at Malta is pasteurized, with the result that Malta fever among them has been reduced 90 per cent, while the disease has not abated among the civil population which continues to use infected milk.

DEFINITION.

Malta fever designates a specific febrile septicemic affection of man characterized by protracted, remittent fever, and is usually associated with anemia, rheumatic pains, and swelling of the joints. It is caused by the *Micrococcus melitensis*.

The manifestations in susceptible animals differ greatly from those in man, and in these the affection may often persist without being noticed.

HISTORY.

The first account of the existence of the disease was furnished by Marston. In 1859 he observed it among the inhabitants of Malta, and gave a complete description of the fever, especially its clinical manifestations. In 1879 Tonaselli made a report of a fatal epidemic of the disease at Catania. Likewise Veale described the fever in patients of Gibraltar, Malta, and Cyprus. The next important step in the knowledge of the disease was reached by the discovery of the causative organism (*Micrococcus melitensis*) by Bruce in 1887.

In 1904 a commission was appointed by the British Government for the purpose of investigating the disease, and very valuable information was obtained from the accurate and careful work of this commission, especially relative to the mode of infection in human beings, this work showing that the goat can be safely incriminated as the principal source of the affection in human beings.

In 1897 Wright established the agglutination test as a suitable diagnostic method for the determination of the disease, and this has constituted the principal procedure for the determination of obscure cases in animals as well as clinical cases in man. A contribution relative to the diagnosis of the disease, especially with reference to the application of the complement-fixation test for the diagnosis of Malta fever, was recently made by the present authors.[1]

OCCURRENCE.

The disease has been endemic on the island of Malta for a very long period, but its presence in other parts of the world has likewise been recognized from time to time, and at the present time its occurrence in tropical and subtropical localities has been noted in almost every country, and quite extensively especially in countries along the Mediterranean. It has been reported from Spain, Gibraltar, Italy, the Mediterranean islands (Balearic Islands, Corsica, Sardinia, Cyprus, Crete, and Grozo), Turkey, China, India, Palestine, the Philippine Islands, North Africa, and South Africa. Of the South American countries, it has been reported from Venezuela, Brazil, Uruguay, Cuba, and Porto Rico.

In the United States the disease has been reported as occurring in the Mississippi Valley. However, authentic cases are not recorded from which the existence of the disease could be considered as positively established. In 1905 Craig reported a case of Malta fever in Washington, D. C., which occurred in a nurse, the disease in this case having probably been contracted from nursing infected soldiers who had returned from the Philippine Islands. He reported 9 cases among soldiers, the infection in these cases probably also originating in the Philippine Islands.

In 1905 the Department of Agriculture imported a number of Maltese goats from the island of Malta for the purpose of obtaining foundation stock for a milch-goat industry in this country. Certain facts in the history of their voyage to this country, which came to our attention about this time, suggested that some of the imported goats were infected with Malta fever virus. The presence of this affection among the Maltese goats was subsequently demonstrated by Mohler and Hart,[2] both bacteriologically and by a series of agglutination tests conducted in this laboratory. As a result of these tests it was deemed advisable to destroy not only the imported goats, which during these investigations were kept in quarantine, but also their offspring, as it was found that even the kids gave positive

[1] Mohler, John R., and Eichhorn, Adolph. A contribution to the diagnosis of Malta fever. Journal of the American Medical Association, Apr. 13, 1912, vol. 58, No. 15.

[2] Mohler, John R., and Hart, George H. Malta fever and the Maltese goat importation. Twenty-fifth Annual Report, Bureau of Animal Industry, 1908, p. 279. Washington, 1910.

reactions to the agglutination test in many instances. At least one person who voluntarily drank the milk of these goats contracted a typical case of Malta fever. Some of the conclusions recorded in this report were the following:

1. It has been definitely demonstrated that the *Micrococcus melitensis*, the organism of Malta fever, has a more or less passive existence in the body of Maltese goats, exercising its pathogenic effect when it gains entrance to the human body.

2. These goats, when carriers of the virus of Malta fever, are one of the important factors, if not the principal factor, in the dissemination of this disease, through the ingestion of their milk by human beings.

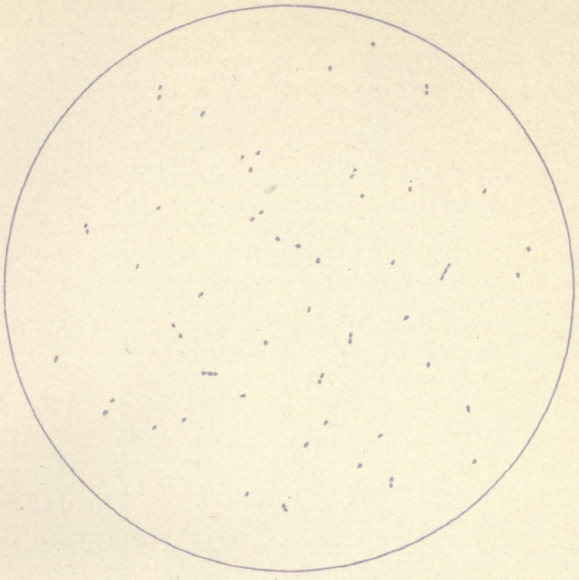
3. Goats infected with Malta fever eliminate the causative agent of the disease in both the milk and the urine.

4. All the available evidence points to contaminated food as the vehicle by which the goats become infected with the organism of Malta fever. The urine of infected goats and of ambulatory cases in man at times contains the *Micrococcus melitensis*, so that normal goats feeding on material which had come in contact with such urine are readily infected. Thus the frequency and the method of infection in goats are quite easily explained.

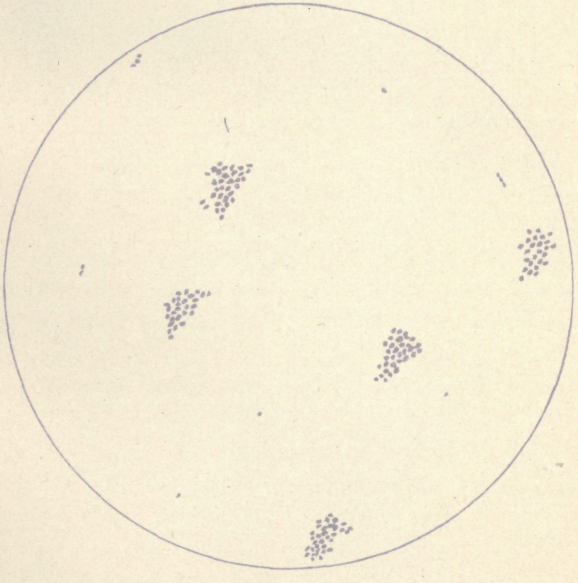
By the recent investigations of Gentry and Ferenbaugh [1] the existence of Malta fever in Texas has been definitely established. Its occurrence in human beings has been demonstrated bacteriologically among certain families in the goat-raising sections of Texas, and since goats have been incriminated as carriers of the infection to man the sera of a number of these animals in the infected localities were subjected to the agglutination test with positive results. The isolation of the *Micrococcus melitensis* from these goats was not successful, and the agglutination test was therefore relied upon for the diagnosis of Malta fever in these animals. The occurrence of the disease in Texas has been substantiated by the writers, who obtained positive results not only with the agglutination test, but also with the complement-fixation test of sera from goats sent to the laboratories at Washington from the infected localities of Texas and New Mexico.

The existence of this disease in Texas is of great moment, inasmuch as the general opinion has prevailed that the United States is free of Malta fever, and that the only occasions when the disease has appeared in this country were isolated instances, occurring through importation. However, from a careful investigation in the infected districts it seems evident that Malta fever, which is also known locally as mountain fever and slow typhoid fever, has existed in Texas and New Mexico for at least 25 years; that the disease has always made its appearance among people connected with goat raising; that entire families have been taken sick with the disease on goat ranches; that many of the goat ranches have had one or more cases

[1] Journal of the American Medical Association, vol. 57, nos. 9, 11, 13, and 14. Chicago, 1911.



1.—Micrococci from bouillon culture 24 hours old. × 1,000.



2.—Agglutination of micrococci with blood serum from Maltese goat. × 1,000.

MICROCOCCUS MELITENSIS, THE CAUSATIVE AGENT OF MALTA FEVER.

(From Twenty-fifth Annual Report.)

BREUKER & KESSLER CO. PHILA.

of the fever among the people connected with them; that in some years there are numerous cases of the disease, while in other years only a few cases will appear. The affection appears usually after the kidding season, during the months of April, May, and June, when the people are in closer contact with the goats. It is stated that the Mexican goat herders are quite infrequently affected, but this may be due not to any natural immunity but to the fact that the Mexicans always boil the milk before drinking it, while the Americans use the milk raw.

The origin of the disease in that section is indefinite, but it is claimed that the affection prevailed in Texas when the common goat was the only goat in the country and long before any of the improved breeds of goats were imported from South Africa, where Malta fever has been found to exist. Most of the goats in the infected districts are of the Angora breed, and none of them were imported from South Africa via Mexico. All the goats imported from Mexico during the last 10 years were the common Mexican goat, almost all of which were brought in for slaughter or grazing purposes through the ports in the lower Rio Grande country.

ETIOLOGY.

The *Micrococcus melitensis* is a very small, round, or slightly oval coccus about 0.4 micron in diameter, occurring singly or in pairs, less frequently in short chains. Involution forms are frequently observed, especially in artificial cultivations. Some of the authors have considered the organism as a short bacillus, which view is principally based on its appearance in stained preparations. In fresh material obtained from infected individuals, however, it invariably appears in the coccus form, and the various involution forms are usually obtained only from older cultures. Capsule and spore formations are never observed.

The *Micrococcus melitensis* is nonmotile and possesses no flagella. In hanging drop preparations it shows an active Brownian movement, which, however, is not true motility.

The *Micrococcus melitensis* may be stained with all ordinary basic anilin dyes, but is Gram negative. Methylene blue and fuchsin give the best results. (See Pl. XIX.)

The organism grows very slowly, even at incubator temperature, and requires a faintly acid medium. The optimum temperature is 37° C.; a slow growth, however, may also take place at room temperature and at 42° C. Under 6° and over 45° C. the growth ceases. The micrococcus is aerobic but may also grow anaerobically.

On agar plates specific colonies appear after 1 to 2 days, which are hardly perceptible to the naked eye. After 2 to 3 days the individual colonies appear as small dewdrops. If the colonies are not very

numerous they may reach a diameter of 1.5 millimeters after 8 days. The single colonies are round, almost spherical; their surface is smooth and shows a uniform granulation. In the center of each colony an oval darker spot is noted which appears most strikingly in the deeper colonies. The colonies of about 4 days' growth are whitish opaque and in a strong light they appear somewhat opalescent. Later the color changes from a deep amber to a pale brown or even darker color.

In stab and also on slant agar the growth at first consists of single colonies; later they coalesce and appear as a moist lustrous growth which at first is yellowish, later becoming a darker color. On gelatin plates at room temperature the growth is extremely slow, so much so that even at the end of the first week the colonies are hardly perceptible to the naked eye. The colonies resemble those on agar plates. The gelatin does not liquefy. In gelatin stabs very small round colonies appear in about 5 days along the entire stab, the colonies close to the surface becoming larger in about 10 days and taking up a yellowish brown color.

In bouillon the growth appears only after the second day, when the fluid becomes cloudy. After 3 days the growth appears more luxuriant in the upper layers than in the lower parts of the tube. After a week a white precipitation forms on the bottom of the tube and continues to increase, and after 4 weeks the greater part of the medium is clear and the deposits increase considerably. The deposit consists principally of shorter and longer chains of the micrococcus. Neutral litmus milk gives a prolific growth in 24 to 48 hours, the alkaline reaction continuing to increase. Litmus milk also gives luxuriant growths. Its consistency is not changed, but the alkalinity develops very rapidly, and the milk takes on a pronounced light-blue color.

Potatoes are not suitable for the cultivation of the organism, as the growth appears as a moist spot hardly perceptible to the eye on the surface of the potato. On alkaline potatoes the growth is stronger, showing after 4 days a light or yellowish deposit.

TENACITY.

Cultures of the *Micrococcus melitensis* kept under favorable conditions will retain their virulence for several months, even for years. Exposed to moist heat at a temperature of 60° C., they die in 10 minutes. It requires a temperature of 90 to 95° C. of dry heat to destroy their virulence. Direct sunlight destroys them in a very short time (one or two hours). In diffused light they resist for seven days. The organism shows considerable resistance against drying. Cover-glass preparations covered with the organism when dried proved active even after 75 days. A solution of 1 per cent carbolic

acid destroys the cocci in 5 to 15 minutes, while a one-half per cent solution of carbolic acid will destroy their virulence in 1 hour. (Eyre.) Pasteurization of infected milk for 20 minutes at 145° F. is sufficient to destroy the organism; therefore milk pasteurized for the destruction of typhoid and tubercle bacilli will contain no living *Micrococcus melitensis*.

PATHOGENICITY.

Experimental inoculations have shown most of the different species of animals to be susceptible to the disease. The affections in the different species, however, are not indicated very perceptibly, and the presence of the disease in some of the animals can be only noted by the continuous elimination of the organism in the secretions and excretions. This applies specially to infections of ruminants, which, while harboring the infection, may pass the organism for months, even years, without manifesting any indications of abnormal health. This fact in itself is of great importance when the control of the disease is considered.

Monkeys inoculated subcutaneously or intravenously with cultures of the *Micrococcus melitensis* will develop a condition which clinically greatly resembles the manifestations of the disease in human beings. After a period of incubation of 2 to 5 days a rise in temperature is noted, which continues to persist for about 10 days. The fever usually is at its height in the afternoon and evening, and toward morning it drops, the animals appearing brightened. Besides the fever, the animals appear depressed and have no appetite, and there are diarrhea and emaciation. After the subsidence of the febrile period a stage without fever follows. This again is followed by a severe febrile attack, which when subsiding usually is followed by recovery. Complications and unexpected death may result at any time during the disease. The autopsies reveal only a swollen spleen of a dark red color, while all other organs appear apparently normal. The micrococcus may be isolated from all organs.

Guinea pigs and rabbits are also susceptible to subcutaneous, intravenous, and intraperitoneal injections, although in some instances they fail to manifest any perceptible symptoms in spite of the continuously progressing emaciation that may extend for weeks or months, depending on the virulence of the organism with which they have been inoculated.

Goats, sheep, cattle, and horses are also susceptible to infections, but in these animals the artificial infection is rarely followed by disturbed general health, although the presence of the disease is indicated by the organisms passed by these animals in the urine, and in females in the milk.

In man accidental infection, sometimes with fatal results, has occurred on a number of occasions among laboratory workers from pure cultures of the organism.

The natural infection is especially of importance in regard to the dissemination of the disease, since the affection in man can only be satisfactorily controlled by a comprehensive knowledge of the modes of infection. It is generally conceded that the milk of goats can be safely incriminated as the principal carrier of the natural infection and as the chief source of infection in man. This is especially substantiated by the fact that the extensive occurrence of the disease among English soldiers stationed at Gibraltar was practically eliminated by prohibiting the consuming of raw goat's milk. Nevertheless, other sources of infection must also be considered, inasmuch as there are authentic cases on record in which the disease appeared in families in which goat's milk had not been consumed in any form, and cases in Texas studied by Gentry and Ferenbaugh indicated in several instances other modes of infection than through goat's milk, although in all instances goats were kept on the premises where the disease occurred in man.

In consideration of the persistence of the *Micrococcus melitensis* in the milk of infected goats for long periods, this source of infection is without doubt the principal mode of dissemination of the disease to man. The dissemination of the disease among animals, however, must be attributed to other sources, and in these cases direct contact, as well as infection through urine and excreta, come into consideration. Examination of the urine from infected animals shows the presence of great numbers of the cocci, and their appearance in the urine continues invariably for the periods in which the organisms are found in the milk. Experiments carried out in this line showed that the urine may be incriminated as the principal source of infection in the propagation of the disease among goats, and probably in those instances where the disease appears in human beings not drinking goat's milk the infection has been accomplished through this medium.

The food and the ground of the premises in which goats are kept are readily contaminated with the urine of infected animals, and in this manner the infection may be contracted by healthy animals either through ingestion of the virus or through inhalation of the dust containing the organism. Artificial experiments conducted in order to establish whether such dried infected dust would be infectious proved positive, although the experiments relative to dust infected with urine from infected animals were negative. The infection may also enter the body through wounds, which was proven by artificial experiments, as it was found that such a mode of infection

is readily possible by the application of small quantities of culture to abrasions on the skin of monkeys.

The principal point of entrance of the organism under natural conditions, however, appears to be through the alimentary canal. This must be considered especially since experience has shown that the most frequent infections which are noted occur from the drinking of infected milk. The experiments which were undertaken in this line by the commission appointed by the British Government offer sufficient conclusive evidence of this mode of infection, as it was shown that one single feeding of goats' milk containing the micrococci even sparingly was sufficient to give rise to the disease. Infection by contact, inhalation, or by biting insects is doubtful and may be almost disregarded, especially in the natural infections, and it is safe to accept the alimentary method of infection as the one great danger by which the disease is propagated.

This having been established, infected goats' milk and probably the swallowing of contaminated dust would come first into consideration as transmitters of the infection, and, as already stated, with the preventive measures directed against the use of goats' milk in infected localities the disease has been controlled to a great extent. To illustrate the effectiveness of such preventive measures it will be sufficient to cite that in the months of July, August, and September, 1905, when the prohibition against goats' milk had not been enacted, 258 cases occurred among the soldiers of Malta, while in the same months in 1906, after the enforcement of this measure, the number fell to 26. Bruce in his work on Malta fever expresses the opinion that nine-tenths of all the cases of Malta fever are due to infection by goats' milk.

ANATOMICAL CHANGES.

The anatomical changes are principally observed at autopsies held on human beings, as well as to some extent on experiment animals, since during the natural course of the disease in animals it is only exceptional that deaths result from the disease. As already stated, animals, especially goats, infected with the disease may harbor the infection for months, even years, without showing the slightest indication of disturbed health. The principal lesion occurring in men or experiment animals dead of the disease is the character of the spleen, which is always enlarged; besides, there may be changes indicative of the febrile stage and of the circulation of toxins in the blood. The lungs may show edema, sometimes with small bronchopneumonic areas. The urine is normal in appearance and color and in some instances may contain albumin. The specific organism is present very frequently in the urine, especially in the protracted

cases. The lymph glands may be swollen, but usually fail to show any characteristic changes.

SYMPTOMS.

From the standpoint of sanitary control of the affection in animals the symptoms offer scarcely any characteristic indications of the presence of the disease in goats or other susceptible domestic animals. The observations of the numerous infections occurring in goats, also in experimentally infected animals, fail to show any signs indicative of the disease. On the other hand, in man there is always a pronounced symptom complex by which the presence of the disease may be suspected and diagnosed.

During the experiments on the diagnosis of the disease in goats carried out by the authors, the artificially infected goats were carefully observed in order to note any changes which might occur. Temperatures were taken twice daily, and the animals were also given careful clinical examinations at frequent intervals. The infected animals showed a rise in temperature of 0.5° to 0.7° C. on the second and third days after the infection, respectively, but outside of that no symptoms whatsoever were noted. The animals appeared in continuous good health, the appetite being normal, and they continued to gain in weight. The successful inoculations of the animals nevertheless were established on the fifth and seventh days respectively after the infection, inasmuch as on those days the first indication of a positive agglutination reaction was obtained. The *Micrococcus melitensis* was also isolated at these times from the blood and urine of those animals.

This appears to indicate the period of incubation after artificial infection. This, however, is by no means the length of time under natural conditions, since in the natural mode of infection much smaller numbers of organisms are taken by the animals, and probably as a result the time of incubation is considerably longer. The period of incubation in human beings is said to range from six days to several months.

Some authors have observed that in affected goats the milk sours shortly after it has been drawn, but this is by no means a constant manifestation in the disease. The most important symptom which is observed among goats affected with Malta fever is the frequency of abortions which result in the course of the disease. Some authors estimate that expulsions of immature fetuses occur in 50 to 90 per cent of the pregnant animals, and abortions in affected animals reoccur also during the succeeding and even at the third gestation following the infection. Abortion is most frequent in the fourth month, but may occur any time from the second month to the full term of pregnancy. If the fetus is carried to the full term it is

usually born dead or dies within a very short time. The symptoms which precede the abortion are not as well marked as those which are noted in cattle during outbreaks of infectious abortion. After aborting the animals recover in the ordinary time, but not infrequently catarrh of the sexual organs, mammatis, and even postpartum infections result.

The symptoms in other animals are likewise most frequently imperceptible, and the presence of the disease can be determined only by the demonstration of the specific organism in the blood, secretions, or excretions.

The symptoms in human beings are usually pronounced and give rise to a more or less severe affection. The most striking symptom is a febrile attack with periods of normal temperatures. The duration of these periods varies considerably during the disease. The fever may be remittent or in other cases intermittent; again it may be continuously high or low, and at all stages of the disease the type of the fever may change. The course of the disease in human beings also varies, as it may be very acute from the onset, the fever setting in with chills and rise of temperature from 40° to 41½° C., associated with severe headache and pain in the back, and a general ill feeling. The pulse and respirations are affected in accordance with the height of the fever, although the acceleration of the pulse is not proportional to the rise of the temperature. Diarrhea may first appear, followed later by constipation. The more frequent subacute form, however, usually commences slower and is more gradual in its development. In this form rheumatic pains in different parts of the body are observed. After a few days from the first indication of disturbed health the evening temperature rises to from 39° to 41½° C., with remissions in the morning. The fall in temperature is always associated with profuse perspiration. The first attack of fever may last from 1 to 5 days, and after an interval of 10 to 14 days with absence of fever a relapse occurs which corresponds to the first attack, except that it is usually of a shorter duration. This condition may continue for a period of 9 months or even longer. Cases have been observed in human beings, however, in which the symptoms were totally absent and the disease was indicated only by the presence of the infective organism in the urine, and likewise by the presence of agglutinins in the blood.

COURSE AND PROGNOSIS.

The course of the disease in animals always appears to be protracted, as cases have been noted in goats when the elimination of the specific organism extended over a period of more than a year. The determination of the disease in infected animals can only be

established by periodical examinations of the blood and urine of the infected animals, the absence of the *Micrococcus melitensis* in such cases being indicative of the absence of the disease. The serum tests could not be considered in this regard, inasmuch as the specific immune bodies may be present in recovered animals for a long time after the subsidence of the infection.

The course in human beings may extend for from six weeks up to a year, and cases have even been observed in which relapses occurred for three years, depending on the acute or chronic character of the disease.

The prognosis in animals is always favorable as far as the health of the animals is concerned. In human beings the mortality is estimated at 3 per cent, deaths usually occurring from a weakness of the heart, or less frequently from hyperpyrexia.

DIAGNOSIS.

With respect to the manifestations of the disease in animals, especially in goats, the clinical diagnosis or even post-mortem diagnosis is almost impossible. In this regard the bacteriological examination may prove satisfactory, although negative results obtained in such instances do not necessarily exclude the possibility of the presence of the disease. Very frequently the animals affected harbor only a small number of cocci, hence bacterioscopical examinations, as well as cultural and test inoculations, may be negative. The isolation of the organism appears to be most satisfactory from the urine and the milk, while the blood does not afford the same opportunities, as it usually contains the organism only in sparing numbers.

When inoculation of test animals is undertaken the diagnosis even in such instances must depend upon the bacteriological examination as well as upon the serum tests of the test animals.

The numerous investigations which have been carried out relative to the methods of diagnosis of Malta fever appear to be quite uniform as to the reliability of the agglutination test for the diagnosis of this malady. Nevertheless, the results of some of the investigators prove that the agglutination value is by no means constant in the suspected patients, and it has further been established that human beings, as well as goats, although apparently recovered from the disease, give an agglutination value of the serum indicative of Malta fever even years after the infection. It has also been found that the agglutination value in the presence of this affection is by no means constant, and occasionally it may even fail to indicate the presence of the infection.

Furthermore, there appears to be a diverse opinion among the investigators of Malta fever relative to the height of the agglu-

tination value which should be considered as indicative of an infection. Thus, by some writers an agglutination of 1:10 is considered as sufficient proof of the presence of the disease; others claim 1:20, while still others require 1:30 as the lowest value for a positive diagnosis. During the quarantine of the goats imported from Malta, which has previously been referred to, the Bureau of Animal Industry conducted a number of experiments in order to determine the agglutination value of normal goat serum for the virus of Malta fever. It was found repeatedly that such sera from healthy goats born and raised at the experiment station gave an agglutination value of 1:40. Hence in the tests of the quarantined Maltese goats an agglutination value of 1:70 within a time limit of 1½ hours was required for a positive diagnosis. Kolle and Hetsch [1] state that the agglutination test is indicative of Malta fever only when its value represents a titre of at least 1:100.

In consideration of this difference of opinion regarding the agglutination value of sera from suspected and infected patients, it was deemed advisable to carry out preliminary experiments to determine whether the complement-fixation test could be utilized for the diagnosis of Malta fever. Sera of a large number of goats from the bureau's experimental farm at Beltsville, Md., were obtained and tested both with the agglutination and complement-fixation tests.[2]

In the meantime two goats were subcutaneously injected with one-half cubic centimeter of a washed agar culture of the *Micrococcus melitensis*.

The agglutination test was applied by both the microscopical and the macroscopical methods, but the best results were obtained from the macroscopical method carried out by a procedure similar to that practiced for the diagnosis of glanders. For this purpose the test fluid is prepared from a 4-days-old glycerin-agar culture of the *Micrococcus melitensis* by heating the culture at 60° C. for 1½ hours, which is then washed with carbolized salt solution, filtered, and diluted to a desired density. This is established by comparative tests with sera of known agglutination values determined by microscopical agglutination. Once the titre of the agglutination fluid has been established by these comparative tests, the proper density of a newly prepared fluid is readily obtained by pouring a sample of each into two beakers of equal size up to the height of about 2 centimeters. The density of the old and new fluids may then be compared by placing the beakers on print, preferably engraved print, and observing the legibility of the print through the fluid. The dilution of the new fluid is continued until the density becomes

[1] Die experimentelle Bakteriologie und die Infektionskrankheiten mit besonderer Berücksicktigung der Immunitätslehre. 1911.

[2] It should be understood that this flock was not infected with Malta fever.

similar to that of the old fluid. The numerous comparative tests which have been undertaken with the microscopical and macroscopical agglutination tests show that a reliable uniformity has always been obtained.

The technique of the macroscopical agglutination test is carried out as follows: The suspected serum is diluted with carbolized salt solution in the proportion of 1 to 40 (0.5 cubic centimeter serum to 19.5 cubic centimeters carbolized salt solution). This constitutes the basic dilution, and from this all the dilutions of sera are made in test tubes in such a way that with the added 2 cubic centimeters of test fluid (bacilli emulsion) the desired dilutions are obtained. Thus, 0.8 cubic centimeter of basic dilution added to the 2 cubic centimeters of test fluid would give a serum dilution of 1 to 100. Any number of different dilutions can be prepared in this manner. The rack containing the test tubes is then placed in the incubator for one-half hour, after which the tubes are removed and centrifugalized for 10 minutes at 1,600 revolutions a minute. The test tubes are then returned to the rack without further incubation and the results read after 1 to 2 hours. The sharply circumscribed lentil-shaped sediment in the center of the bottom of the test tube with cloudiness of the upper portion of the fluid indicates the failure to agglutinate, while an irregular veil-like clumping of the sediment over the bottom of the tube with a clearing of the upper part of the fluid is indicative of agglutination. The racks are so constructed that they have a conical opening on the lower shelf into which the bottom of the test tubes fit, and through these openings the reaction is plainly visible, especially when placed on a dark background. A more detailed description of the technique of this method as applied to glanders, together with drawings showing the above-described appearances of the sediment in both positive and negative reactions, will be found in an article in the Twenty-seventh Annual Report of the Bureau of Animal Industry for 1910, pages 345 to 370, reprinted separately as Circular 191.

This method of agglutination would come especially into consideration if large numbers of sera were to be tested, and particularly if it were desired to examine the blood of a great number of goats in certain localities for the control or possible eradication of the disease.

The sera of healthy goats from the experimental farm and that of 20 suspected goats from Texas and 20 similar goats from New Mexico, as well as the sera from the artificially infected animals, were carefully tested by the microscopical agglutination method and also by the method just described. The results showed practically no variation in these two methods, and in consideration of the simplicity of the macroscopical method as described it would appear

that it should be given the preference over the microscopical method, especially in cases where a large number of individuals are to be tested.

In the meantime experiments have been conducted with the complement-fixation test for the diagnosis of Malta fever with sera from normal as well as from infected animals. The hemolytic system consisted of sensitized rabbit serum, serum from a guinea pig, and a 5 per cent suspension of washed sheep corpuscles. An antigen was prepared from 4-days-old glycerin-agar cultures, and after heating for 2 hours at 60° C. it was agitated for 4 days in a shaking machine. The extract was then placed in centrifuge tubes and centrifugalized for 2 hours at a speed of about 2,500 revolutions a minute. The clear fluid was drawn off and preserved with 10 per cent of a 5 per cent carbolic-acid solution. A titration of the antigen was then undertaken in order to establish the smallest quantity which would no longer prevent hemolysis. A dilution of 1:50 was found to be the proportion of antigen to be used in the tests.

The goat serum to be tested was inactivated at 56° C. for 30 minutes. The complement was titered in each instance in order to establish the necessary smallest quantity required to produce complete hemolysis. Of the sera to be examined, 0.2 and 0.1 cubic centimeter, respectively, were used, and the customary control tubes were always included in the test. Thus, in routine testing, four tubes were taken for the test proper, the first pair receiving 0.1 cubic centimeter of serum and the second pair 0.2 cubic centimeter. The second and fourth tubes served as controls for the serum, in order to establish that the serum without the antigen would not produce a fixation of complement.

The sera from the goats at the experimental farm failed to give a fixation in any instance, although in several cases it was observed that the hemolysis resulted slowly, and sometimes a very small quantity of blood corpuscles settled to the bottom of the tube, but in all these instances the reaction was the same in all four tubes; namely, in the tubes for the test proper, as well as in the control tubes. Of the sera examined from the suspected cases sent from Texas, 4 gave positive complement fixation, and in all these instances the fixation was complete, even in the tube in which only 0.1 cubic centimeter of serum had been used, while 16 other examinations of sera from the same source gave negative results.

The agglutination test of the sera from the cases in which a fixation of the complement was obtained showed a value in one instance of 1:50, in two 1:15, and in the fourth it failed to agglutinate even at 1:10. The remaining 16 cases failed to agglutinate in the proportion of 1:10.

In the tests made with the sera of the New Mexican goats only one case gave a positive reaction to the complement-fixation test.

The sera of the goats which had been artificially infected were drawn every day from the time of infection and examined both by the agglutination and the complement-fixation tests. On the fifth day an agglutination of 1:40 was obtained, which on the subsequent days was marked at 1:500 and reached a height of 1:2,000, continuing to give an agglutination value of over 500 for the two months after infection, during which period the animals have been under constant observation. A partial complement fixation was first obtained on the seventh and ninth days, respectively, and from that time on a perfect fixation was obtained in all instances. In these tests even smaller quantities than 0.1 cubic centimeter gave a fixation; thus, in establishing what would be the smallest quantity of serum which would give a complete fixation, it was found that on the twenty-second day after the infection in one goat 0.04 cubic centimeter of serum gave a complete fixation.

From the results of these investigations it appears that the complement-fixation test can be utilized for the diagnosis of Malta fever, and in consideration of the fact that the agglutination test is not always reliable for such purposes the complement fixation would be of great advantage as an adjunct in the diagnosis of this malady.

PREVENTION AND TREATMENT.

Since the nature of the disease was established attention has been directed toward establishing immunity by serum treatment as well as by vaccines. The investigations in this line on test animals have shown that test animals inoculated with dead organisms fail to give protection to subsequent virulent infections.

Wright aimed to produce a horse serum by treating goats and horses with *Micrococcus melitensis*. About 50 cases were treated with such horse serum, but the published results do not offer conclusive proof of the effectiveness of this serum.

The present authors have also experimented on infected goats with a vaccine prepared along the lines of antityphoid vaccine. The goats were injected four times at intervals of one week with such vaccine containing increasing numbers of killed bacilli. The blood was drawn from time to time from these animals, and cultures were also made at different times from the urine and the blood. The results showed that during the time of treatment the urine still contained virulent organisms. The serum tests both with the agglutination and complement fixation continued to give positive results, although four weeks after the last vaccine injection no bacilli could be longer demonstrated by culture or other methods.

The practical value of the vaccine treatment is very doubtful, inasmuch as it would be almost impossible to isolate strictly all animals giving a positive serum reaction and subject them to a vaccine treatment. Therefore it appears that hygienic and preventive measures are of far greater value in the control and possible eradication of the disease.

The control of the disease in a herd could be best accomplished by subjecting the blood serum of all animals to the combined agglutination and complement-fixation tests and destroying all reacting animals. Further, care should be taken to have all newly purchased animals tested and permit their introduction into the herd only if the absence of the disease has been established. However, even the strictest measures in this direction would give no assurance of a positive safeguard against the introduction of the disease, as the virus may be carried by other means than by goats, such as dogs and rabbits.

Measures for the control of the disease would, of course, have to be adopted in accordance with the extent of the infection. Should the serum tests indicate only a moderate spread of the disease in a locality, measures for eradication could be adopted by killing all animals giving a positive serum test. On the other hand, such a procedure could hardly be considered in localities where the disease affects a large percentage of the animals, as, for instance, among the goats on the island of Malta. In such cases the problem of eradication would be very difficult, and the infection of man could be best guarded against by the pasteurization of all goats' milk and the removal of the corrals and kidding pens from proximity to dwellings. The sanitary conditions around the goat ranches should be improved. The goat pens as a rule are very close to the ranch house (see Pls. XX and XXI), and the goat manure has been accumulating in these pens ever since goats have been kept therein. Frequently the goat bedding ground is around the yard fence. The water supply is usually from wells and small streams, and in many instances these may readily become contaminated from the goat pens.

As in the case of all other infectious diseases, thorough disinfection of the infected corrals, pens, and utensils should be carried out in order to prevent the recurrence of the disease. As a disinfectant the compound solution of cresol, carbolic acid, or chlorid of lime may be used by mixing 6 ounces of any one of these chemicals with 1 gallon of water. One of the approved coal-tar sheep dips might also be used to advantage in a 5 per cent solution (6 ounces of dip to 1 gallon of water). After removing the manure and litter the disinfectant solution should be applied liberally to all part of the pens, and sufficient lime may be added to the solution to make the disinfected area conspicuous.

In localities where the disease is prevalent an educational campaign dealing with the necessity of heating the milk, as is done for the prevention of milk-borne typhoid fever, would greatly aid in the prevention of infection in man.

No thorough investigations have yet been undertaken as to the extent of Malta fever among the goats of Texas, New Mexico, and possibly in other States, and until this is determined it is impossible to decide upon a definite line of procedure for the control and eradication of the disease.

Although the disease has no active effect on goats, its eradication must be considered from the standpoint of public health, and in this respect it is of the highest importance, since there is a tendency at the present time among physicians to advise the drinking of goats' milk for children and invalids.

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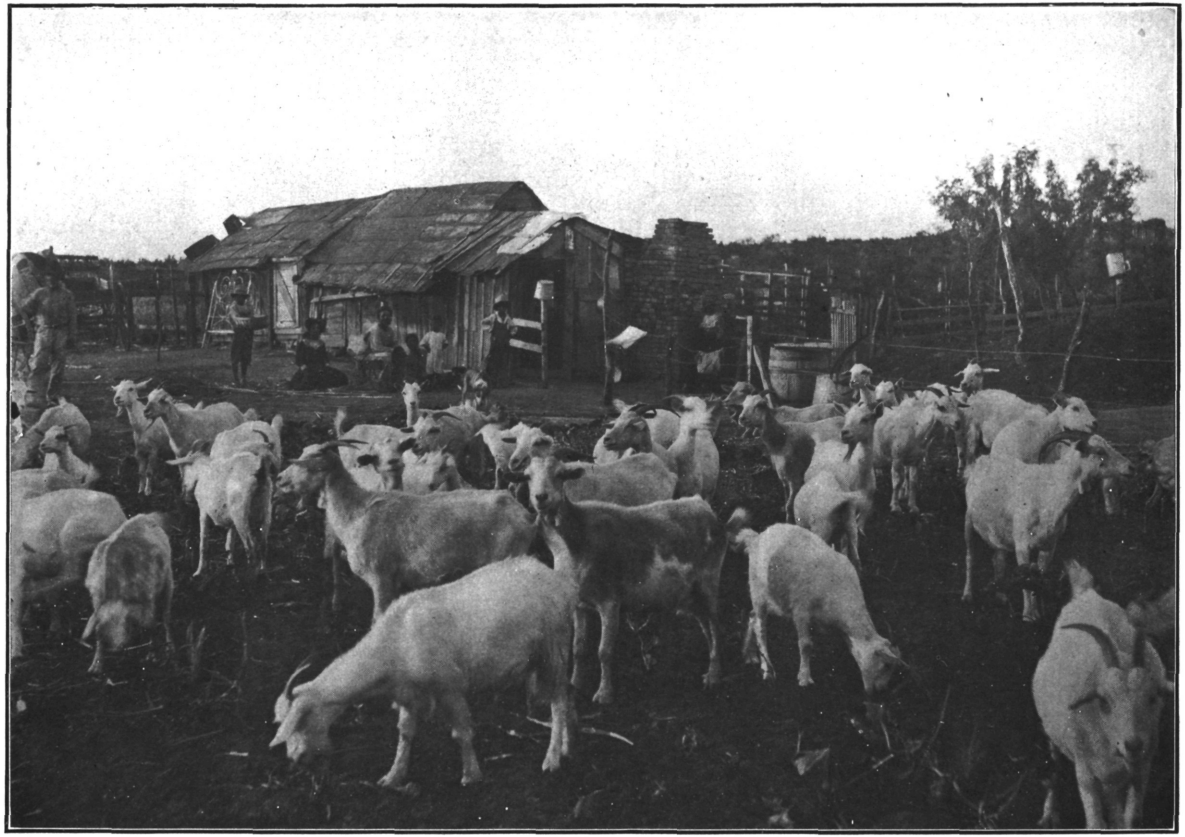
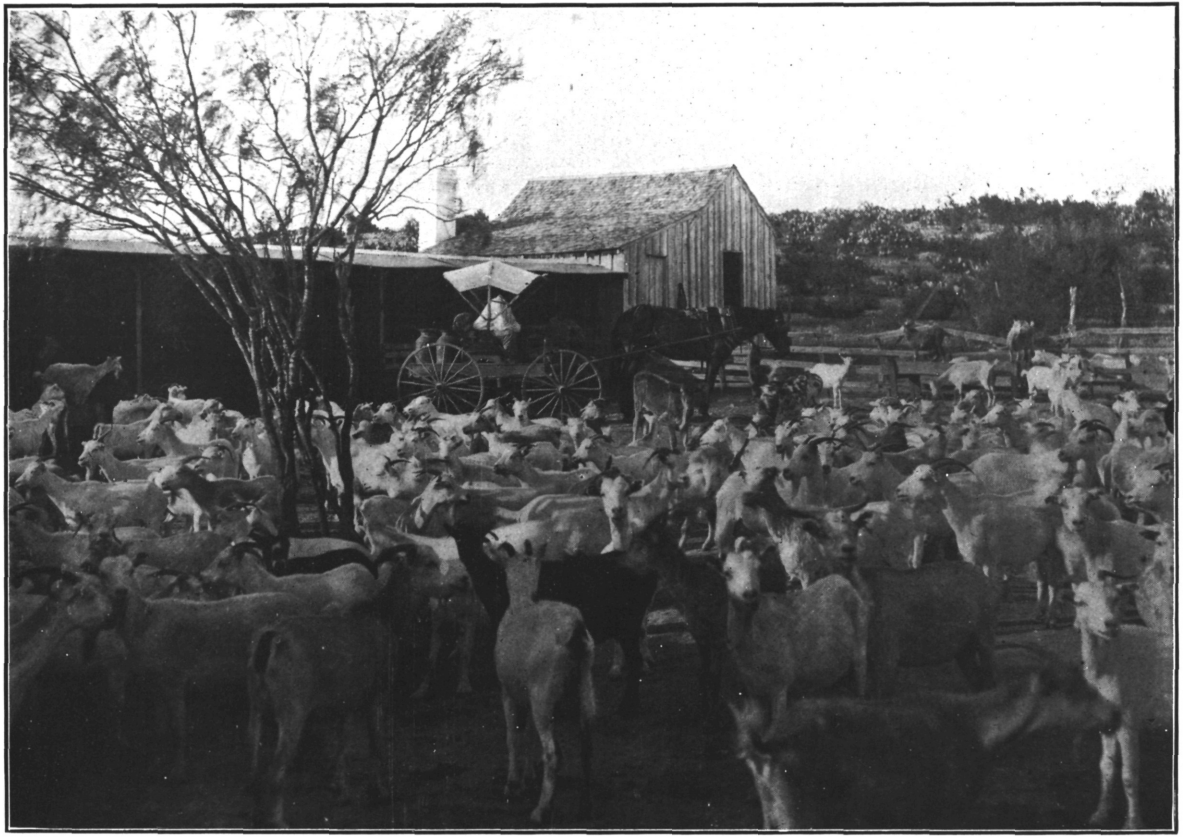


A HERD OF MILCH GOATS IN TEXAS, SHOWING PROXIMITY OF BED GROUNDS TO DWELLING.



A HERD OF MILCH GOATS IN TEXAS, WITH PENS ADJACENT TO OWNER'S HOME.

A HERD OF 600 MILCH GOATS IN TEXAS, WITH DAIRY IN THE BACKGROUND.